22. (Amended) A method for refolding an insulin-like growth factor-I (IGF-I) polypeptide derived from a yeast cell medium to yield an authentic, properly folded IGF-I polypeptide comprising denaturing and renaturing IGF-I species present in an IGF-I mixture from said yeast cell medium using an unfolding/refolding buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions that allow for the reduction and subsequent oxidation of disulfide bonds, thereby producing an authentic, properly folded IGF-I polypeptide.

23. (Amended) The method of claim 22 wherein the denaturing and renaturing are performed together using an unfolding/refolding buffer comprising about 1.5 to about 3 M urea, about 1 mM to about 15 mM sodium borate, about 1 M to about 3 M sodium chloride, about 5% to about 20% ethanol, and about 0.005 mM to about 10 mM dithiothreitol.

24. (Amended) The method of claim 23 wherein the unfolding/refolding buffer has a pH of about 8.5 to about 10 and comprises about 2 M urea, about 5 mM sodium borate, about 1.5 M sodium chloride, about 15% ethanol, and about 0.2 mM dithiothreitol.

25. (Amended) The method of claim 24 wherein the unfolding/refolding buffer has a pH of about 9.0 to about 9.5.

REMARKS

Claims 1-26 are pending in the present application. Claims 1 and 22-25 have been amended herein. No new matter has been added. Upon entry of the present amendment, claims 1-26 will be pending. Because the amendments to the claims remove an issue for appeal (*i.e.*, indefiniteness), Applicants respectfully request that they be entered into the record. See, M.P.E.P. § 714.12. In addition, the amendments to the claim 1 are identical to those amendments entered by the Examiner in the parent application in response to the same indefiniteness rejection.

As a preliminary matter, Applicants acknowledge receipt of the "Attachment for PTO-948" outlining changes for prosecution of applications containing drawings. Formal drawings have been filed on date even herewith under separate cover to the Draftsperson.

The first line specification has been amended to contain the claim for priority under 35 U.S.C. § 120. The claim for priority was originally made at page 2 of the transmittal of the as-filed application.

Claim 1 has been amended to recite the invention with greater particularity -- *i.e.*, language objected by the Examiner has been replaced with alternative language that was accepted by the Examiner in the parent application. In addition, claims 22-25 have been amended to replace "denaturing buffer" with "unfolding/refolding buffer," support for which can be found, for example, at page 12, lines 14 to 29 of the specification. No new matter has been added.

I. Obviousness-Type Double Patenting

Claims 1-26 are rejected under the doctrine of obviousness-type double patenting over claims 1-21 of U.S. Patent No. 5,650,496 and claims 1-25 of U.S. Patent No. 6,207,806. Applicants enclose herewith a Terminal Disclaimer for each of the above-recited patents. Accordingly, the obviousness-type double patenting rejection is moot.

II. The Claims Are Clear And Definite

Claims 1-26 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Applicants traverse the rejection and respectfully request reconsideration thereof because the claims are clear and definite.

The Office Action mistakenly asserts that the phrases "a substantial amount" in claim 1, step (b), "a significant proportion" in claim 1, steps (f), (i), (l) and (m), "a substantial proportion" in claim 1, step (l), are vague and indefinite because the skilled artisan is not able to determine what is within the scope of the claims. Although Applicants submit that these phrases are sufficiently clear and definite, they have been deleted from the claims and replaced with alternate language to advance

prosecution of the present application. No change in claim scope is intended. It is to be understood that the wording of the present claims is not intended to mean that, for example, *complete* removal of unwanted molecules is achieved while *complete* retention of desired IGF-I molecules is accomplished, as it is readily apparent that 100% retention and removal of unwanted molecules and IGF-I molecules is not always possible. In addition, the amendments to claim 1 to replace the objected language are identical to the amendments accepted by the present Examiner in the parent application.

The Office Action also asserts that recitation of "in sufficient amounts and under conditions that allow" renders claim 22 indefinite. Applicants respectfully point out that the description of the invention is the role of the specification, not the claims. *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 1 U.S.P.Q.2d 1081 (Fed. Cir. 1986). The amount of detail required to be included in the claims is not to be viewed in the abstract but in conjunction with the specification. *Shatterproof Glass Corp. v. Libbey-Owens Ford Co.*, 225 U.S.P.Q. 634 (Fed. Cir. 1985). As such, the specification contains numerous examples of concentrations of the buffer components. Further, claim 22 recites that the buffer components are present in sufficient amounts to produce an authentic, properly folded IGF-I polypeptide. M.P.E.P. § 2173.05(c) states, in section III titled "EFFECTIVE AMOUNT":

> The phrase 'an effective amount ...for growth stimulation' was held to be definite where the amount was not critical and those skilled in the art would be able to determine from the written description, including the examples, what an effective amount is. *In re Halleck*, 422 F.2d 911, 164 USPQ 647 (CCPA 1970).

In view of the teachings in the specification, one skilled in the art would be able to determine an amount of each of the buffer components sufficient to produce an authentic, properly folded IGF-I polypeptide. ***No reasoning or evidence to the contrary is provided in the Office Action.***

In addition, one skilled in the art, having examined Applicants' specification, would be able to determine conditions that allow for the reduction and subsequent oxidation of disulfide bonds, thereby producing an authentic, properly folded IGF-I polypeptide. Applicants' specification provides ample description of conditions that allow for the reduction and subsequent oxidation of disulfide bonds, thereby producing an authentic, properly folded IGF-I polypeptide. For example,

page 12, line 22 to page 13, line 31 of the specification teaches components, concentrations, pHs, time courses, and temperature -- *i.e.*, conditions -- that allow for the reduction and subsequent oxidation of disulfide bonds of IGF-I polypeptides. Thus, the claims comply with § 112. *In re Mercier*, 185 U.S.P.Q. 774 (C.C.P.A. 1975) (claims sufficiently define an invention so long as one of ordinary skill can determine what subject matter is or is not within the scope of the claims). Accordingly, Applicants respectfully request that the rejection of claims 1-26 under 35 U.S.C. § 112, second paragraph be withdrawn.

Claims 22-26 are also rejected under 35 U.S.C. § 112, second paragraph, as allegedly being incomplete for omitting essential steps. Applicants traverse the rejection and respectfully request reconsideration thereof because the claims are complete.

The Office Action mistakenly asserts that the following steps are essential, but nonetheless, omitted from the claims: 1) isolation of the IGF-I polypeptide from the yeast cell medium; 2) addition of the denaturing agent; 3) separation of the non-denatured from the denatured; and 4) separation steps for removing the folded IGF-I from the unfolded IGF-I. No other omitted steps are identified in the Office Action. The Office Action, however, provides no reasoning, let alone evidence, supporting the conclusion that the steps recited therein are essential to the claimed invention. The Examiner is reminded that claims 22-26 recite, in part, methods for "***refolding*** an insulin-like growth factor-I (IGF-I) polypeptide derived from a yeast cell medium to yield an authentic, properly folded IGF-I polypeptide" (emphasis added). Indeed, there is no evidence of record indicating that the steps identified in the Office Action are required to be recited in claims 22-26 to carry out the claimed methods of "refolding" IGF-I.

The methods recited in claims 22-26 do not require "isolation of the IGF-I polypeptide from the yeast cell medium." As stated above, the claims recite methods of "refolding" IGF-I, not isolating IGF-I. In addition, claim 22 recites that the IGF-I is "present in an IGF-I mixture from said yeast cell medium." Thus, IGF-I need not be isolated from the yeast cell medium to carry out the claimed methods. The Office Action provides no reasoning or evidence to the contrary.

The methods recited in claims 22-26 also do not require "separation of the non-denatured from the denatured" IGF-I or "separation steps for removing the folded IGF-I from the unfolded IGF-

I." Again, as stated above, the claims recite methods of "refolding" IGF-I, not separating denatured from non-denatured IGF-I or separating folded from unfolded IGF-I. Thus, IGF-I need not be separated based upon its folding status or denaturing status to carry out the claimed methods. The Office Action provides no reasoning or evidence to the contrary.

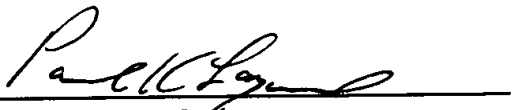
To the extent that the methods recited in claims 22-26 require "addition of the denaturing agent," claim 22 recites "denaturing ... IGF-I species ...using an unfolding/refolding buffer comprising urea, dithiothreitol, alcohol and salt." Urea and dithiothreitol are, indeed, denaturing agents. Thus, claims 22-26, in fact, recite denaturing IGF-I using a denaturing agent. The Office Action provides no reasoning or evidence to the contrary.

Thus, in view of the foregoing, Applicants respectfully request that the rejection of claims 22-26 under 35 U.S.C. § 112, second paragraph be withdrawn.

III. Conclusion

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 564-8906 if there are any questions regarding Applicants' claimed invention. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

At page 1, after the title, the following paragraph has been inserted:

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. Serial No. 08/851,162 filed May 5, 1997, which is a divisional of U.S. Serial No. 08/422,436 filed April 14, 1995, each of which is incorporated herein by reference in its entirety.

In the Claims:

Claims 1 and 22-25 have been amended as follows:

1. (Amended twice) A process for purifying correctly folded monomeric insulin-like growth factor-I (IGF-I) from a medium containing IGF-I peptides, comprising the steps of:

(a) contacting the medium with a sufficient quantity of a first cation exchange matrix under conditions allowing adsorption of at least about 95% of total IGF-I from the medium;

(b) washing the IGF-I-loaded first cation exchange matrix with a first cation exchange wash buffer, which removes [a substantial amount of] adsorbed non-IGF-I material [without removing a substantial amount] <u>from said matrix and allows retention</u> of authentic or non-authentic IGF-I <u>by said matrix</u>;

(c) eluting all forms of adsorbed IGF-I from the cation exchange matrix of step (a) by contacting said cation exchange matrix with a sufficient quantity of a first cation exchange elution buffer, which has a sufficiently high pH or ionic strength to displace substantially all of said authentic and non-authentic IGF-I from said cation exchange matrix;

(d) transferring the IGF-I-containing eluate from step (c) into an unfolding/refolding buffer, which:

(i) reduces the intrachain disulfide bonds of IGF-I protein and promotes unfolding without permanent denaturation; and

(ii) permits refolding of the IGF-I and reoxidation to form properly-paired intrachain disulfide bonds;

(e) contacting the properly folded IGF-I from step (d), after transfer into a suitable solvent system, with a sufficient quantity of a hydrophobic interaction chromatography matrix under conditions allowing adsorption of at least about 95% of said IGF-I from said eluate;

(f) washing the IGF-I-loaded hydrophobic interaction chromatography matrix with a hydrophobic interaction wash buffer having an ionic strength sufficiently low to remove most of the non-authentic IGF-I[, but not so low as to remove a significant proportion of the authentic IGF-I] from the hydrophobic interaction chromatography matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix;

(g) eluting the adsorbed IGF-I from said hydrophobic interaction chromatography matrix by contacting said matrix with a hydrophobic interaction elution buffer, which has a sufficiently elevated pH, or sufficiently low ionic strength, to cause displacement of substantially all of the adsorbed authentic IGF-I from said matrix;

(h) contacting the eluate from step (g) with a sufficient quantity of a second cation exchange matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(i) washing the IGF-I-loaded second cation exchange matrix with a cation exchange wash buffer having a sufficiently high ionic strength, or sufficiently high pH, to remove [a significant proportion of] non-authentic IGF-I[, but not so high as to remove a significant proportion of authentic IGF-I] from said matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix;

(j) eluting the adsorbed IGF-I from said second cation exchange matrix by contacting said matrix with a second cation exchange elution buffer, which has a sufficiently high ionic strength, or sufficiently high pH, to displace substantially all of the adsorbed authentic IGF-I from said matrix;

(k) contacting the eluate from step (j), in an aqueous buffer, with a suitable quantity of a reverse phase chromatography matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(l) washing the IGF-I-loaded reverse phase chromatography matrix with an aqueous/organic reverse phase wash buffer having an organic solvent concentration sufficiently high to remove [a

substantial proportion of] non-authentic IGF-I[, but not so high as to remove a significant proportion of authentic IGF-I] from said matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix; and

(m) eluting the adsorbed IGF-I from said reverse phase chromatography matrix with an aqueous/organic buffer having an organic solvent concentration high enough to remove substantially all of the authentic IGF-I without removing [a significant proportion] substantially all of the multimeric forms of IGF-I from said matrix.

22. (Amended) A method for refolding an insulin-like growth factor-I (IGF-I) polypeptide derived from a yeast cell medium to yield an authentic, properly folded IGF-I polypeptide comprising denaturing and renaturing IGF-I species present in an IGF-I mixture from said yeast cell medium using [a denaturation] an unfolding/refolding buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions that allow for the reduction and subsequent oxidation of disulfide bonds, thereby producing an authentic, properly folded IGF-I polypeptide.

23. (Amended) The method of claim 22 wherein the denaturing and renaturing are performed together using [a denaturation] an unfolding/refolding buffer comprising about 1.5 to about 3 M urea, about 1 mM to about 15 mM sodium borate, about 1 M to about 3 M sodium chloride, about 5% to about 20% ethanol, and about 0.005 mM to about 10 mM dithiothreitol.

24. (Amended) The method of claim 23 wherein the [denaturation] unfolding/refolding buffer has a pH of about 8.5 to about 10 and comprises about 2 M urea, about 5 mM sodium borate, about 1.5 M sodium chloride, about 15% ethanol, and about 0.2 mM dithiothreitol.

25. (Amended) The method of claim 24 wherein the [denaturation] unfolding/refolding buffer has a pH of about 9.0 to about 9.5.